Original Research Article

Pharmacognistic, Acute Toxicity profile and comparative leaf characteristics of *Desmodium velutinum*, *Mallotus oppositifolius* and *Synedrella nodiflora*.

Abstract

Plant leaf parameters such as stomatal index, stomata type, leaf microscopic features and leaf powder microscopy are important taxonomic parameters used to properly identify specie and to differentiate one specie to another and this are vital key in pharmacognostic study of medicinal plant because leaf parameters play a vital role in distinguishing crude drugs even when they are fragmented or powdered. Also, extractive index, retention factor, ash value and phytochemical parameters are vital in standardization of crude drug for establishment of pharmacopeia and monograms. Therefore, the study seeks to validates the use of the phytochemistry and molecular systematics among Asteraceae, Euphorbiaceae and Fabaceae to support their circumscription and therefore evaluated the pharmacognistic profile of *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* folklorically used in South Eastern Nigeria using standard laboratory protocols. The result of the leaf microscopy showed that *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* all had irregular shaped leaf epidermis and stomata was present only in the abaxial surface respectively. The result showed both *D. velutinum*and *S. nodiflora*powder showed the presence of lignin, starch, Cystoliths, tannin and Oil body while calcium oxalate were absent whereas, *Mallotus oppositifolius* showed the presence of lignin, starch, tannin and Oil body while calcium oxalate and Cystoliths were absent. Also the result of the TLC showed that *M. oppositifolius* had 3 visible spot with retention factor (RF) of 0.363, 0.425 and 0.5 in that order respectively, *D. velutinum* had 4 visible spot with retention factor (RF) of 0.425, 0.5, 0.550 and 0.66 in that order respectivelywhile *S. nodiflora* had 6 visible spot with retention factor (RF) of 0.255, 0.363, 0.438, 0.538, 0.600 and 0.688 in that order respectively. The result also showed that methanol exhaustible extracted the phytochemicals in the crude dried plant. The acute

toxicity studies showed that *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* individually had LD50 greater than 5000mg/kg. This study validates the claim that leaf parameters such as stomatal index, stomata type, leaf powder microscopy are important parameters used for identification of crude drugs.
Key words: Pharmacognistic, Acute Toxicity, *Desmodium velutinum*, *Mallotus oppositifolius*, *Synedrella nodiflora*, leaf parameters**.**

Introduction

Though the use of herbs as crude drug is as old as man's existence on earth, its proper identification is very important to its continued benefits. Herbal drugs with high and effective therapeutic activity usually witness surge in demand and this therefore sometimes leads to its adulteration, replacement and abuse by the herbal practitioners and marketers of herbal drug leading to consumers disappointment with numerous fatal consequences [1] and even mortality.The increased demand and use of herbal drugs represent a substantial proportion of the global drug market [2-8] due to its perceived efficacy, acceptability and safety. Adulteration of drugs with different materials is usually done by traditional medicine practitioners in order to increase the economic gain and this can lead to public health challenge as a result of varied level of toxicities. There is continued emphasis on proper identification, collection, isolation and screening of plants for biological activities in order to authenticate their safety and therapeutic claims. Accurate identification, collection and preparation of herb for evaluation and for therapeutic administration are of high importance in drug discovery and development.

Whereas, it is generally believed that most herbal preparations are safe for consumption, some herbs and certain herbal formulations could be toxic with undesirable side effects [9] Inaccurate identification or collection of plants for therapeutic activity can be catastrophic and may even lead to waste and mortality. Problems of improper identification of crude drug, adulteration and toxicities can be solved by proper pharmacognistic studies of medicinal plants which is essential in drug standardization and formulation. This thereby, will reduce concerns of inaccurate identification, collection and toxicities.

The leaf parameters such as stomatal index, stomata type, leaf microscopic features and leaf powder microscopy are important taxonomic parameters used to properly identify specie and to differentiate one specie to another.

The stomata apart from their roles in transpiration and gaseous exchange are very vital tool found very useful as diagnostic tools in plant taxonomy and systematic [10]. Also, the leaf micro-characters such as the stomatal type, stomata size, types of cell wall thickness, stomata index and occurrence are useful in taxonomic distinction and recognition in the flowering plant families [11] Leaf parameters are also a vital tool for distinguishing crude drugs even when they are fragmented or powdered.

Therefore, the study seeks to validates the use of the phytochemistry and molecular systematics among Asteraceae, Euphorbiaceae and Fabaceae to support their circumscription.

Desmodium velutinum, *Synedrella nodiflor*a and *Mallotus oppositifolius* areimportant botanicals used throughout the southern Nigeria for the treatment of various diseases conditions ranging from infections, wounds, ear ache, ulcer and feverand therefore this study tried to evaluated the pharmacognistic profile of this three Nigerian plants. Desmodiumvelutinum (*D. velutinum)*belongs to the botanical family Fabaceae. The plant is generally called Ikeagwani and the leaves are used to control non- specific diarrhoea among the people of the south eastern Nigeria. Extracts of *Desmodium velutinum* are used traditionally in some disease conditions particularly headache, fever and diarrhoea [12]. The squeezed leaves of *D. velutinum* is locally used for diarrhoea while the stem and root are chewed to relieve tooth ache [12]. Desmodium velutinum is a perennial, erect or semi-erect shrub or sub-shrub, up to 3 m high. The branches are often dark red or yellow-brown when young, velutinous and short hooked-hairy.

Synedrella nodiflora (L.) (*S. nodiflora*) Gaertn is of the botanical family Asteraceae, ethnobotanically known for wound healing. It is a small, annual weed of cultivation native to America, found in the plains of India and in the Andamans also. The leaves are used as poultice for sore rheumatism and juice of the leaves is used for earache

[13]. In Ghana the infusion of leaves of *S. nodiflora* is taken as laxative; leaf sap is used in Cango for mouth affections and is rubbed on gums for tightening.

Mallotus oppositifolius (Geiseler) Mull. Arg. (Euphorbiaceae) is a predominant edible shrub in the South-Eastern – Nigeria where it is commonly identified as Ukpo [14]. It is used in Nigerian folk medicine for the treatment of common infections caused by bacteria, fungi pathogens and for the treatment of wounds and ulceration. It has been reported that *M. oppositifolius* exerts antifungal properties [15-16] against pathogens of public health importance

This present study evaluated the anatomical and chemical profile of *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* found in south eastern Nigeria.

Materials and methods

Test Plant Collection

The three plants studied are of public health importance and their selection for studies is because of their therapeutics (biological) and economic importance. The study compared the pharmacognostic and phytochemical profile of Asteraceae, Euphorbiaceae and Fabaceae. Fresh leaves of *M. oppositifolius*, *Desmodium velutinum* and S*ynedrella nodiflora* were collected from their natural habitat around Anambra State South Eastern Nigeria. The plant was properly identified by a plant taxonomist Mr. Felix Nwafor at the Department of Pharmacognosy and Environmental Medicine University of Nigeria Nsukka and the voucher specimen was prepared and deposited at the herbarium department of University of Nigeria Nsukka with Voucher number PCG/UNN/0336/Fabaceae PCG/UNN/0337/Euphorbiaceae, PCG/UNN/0338 Asteraceae. The plant leave was air dried at room temperature (25 ^{o}C) for 14 days to obtain the powder for phytochemical and physicochemical parameters.

Fresh Leaf Microscopy

Leaf microscopy to determine the types of epidermal cells, stomata type, stomata size, stomata density, presence of trichome, vein islet number and stomata index was on both the adaxial and abaxial surfaces of the leaves were carried out using the methods of [17]. Transverse section (TS) of the leaf was made using a Reichert sledge microtome following the procedures of [18-19].

Powder Microscopy / Chemo-microscopy

The leaves were dried under shade and pulverized with local mortar and pestle. Chemo-microscopy conducted on the powders to determine the presence of starch, calcium oxalate crystals and lignified vessels using standard laboratory procedures according to [20-21].

Extractive Value

The extractive value for methanol, water, and n-hexane was determined by the method of the method of[20-21]

MOISTURE CONTENT

Moisture content of the three plants were determined by the method of [20-21].

Total Ash Value

The total ash, water soluble ash and acid soluble ash value were determined by the method of [20-21].

Phytochemical Screening.

The qualitative Phytochemical analysis was done using the method of by[20-23] to determine the presence of secondary metabolites or active components in *Mallotus oppositifolius*, *Desmodium velutinum* and

Synedrellanodiflora.Estimation of the amount of phytochemicals present was assayed by using standard procedures described by [24-25].

Acute Toxicity Studies

The acute toxicity test to determine the LD_{50} as an index of safety of the extract was done in 2 phases as described by [25] for *M*. *oppositifolius* as described by [26]. Briefly, nine animals (rats) were randomly allocated into 3 groups of 3 rats each. Animals in group 1, 2 and 3 were given 10, 100, and 1000 mg/kg body weights respectively of the extract through the oral route. Animals were therefore monitored for signs of toxicity and mortality for 2 days (48 hours). Signs of toxicity and pathological findings observed were recorded appropriately. All the animals survived so the extract and was further subjected to acute toxicity test with higher doses in the second trial. In the second trial, 4 animals were randomly allocated to 4 groups of one animal each. Animals in group 1, 2, 3 and 4 were given 1200, 1600, 2900 and 5000 mg/kg body weight, respectively of the extract.

Result and Discussion

Leaf Microscopy and Leaf Characteristics

The result of the fresh leaf microscopy is presented in Plate 1 to 9 and Figure 1 while the leaf powder is presented in figure 10-12 and Table 1. Leaf of the plant were studied for leaf type, epidermal cell type, stomata type, stomata

density, epidermal cell number, stomata index, stomata length, stomata width, stomata size. Palisade ration and the powder of the ground leaf was evaluated for powder microscopy to evaluate the presence of lignin, starch, calcium oxalate, tannins etc.

Qualitative Leaf Microscopy*Desmodium velutinum, Mallotus oppositifolius* and *Synedrella nodiflora*

The result of the leaf microscopy is presented in plate 1 to 9. The result showed that the upper (adaxial) and lower (abaxial) leaf epidermal surface of *Desmodium velutinum* are irregular in shape with undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. The leaf is hypostomatic which implied that stomata only occur on the lower surface of the leaf. The stomata are paracytic which implied that two subsidiary cells positioned parallel to the guard cells. There is also presence of unicellular, unglandulartrichome with bulbous base while in *Mallotus oppositifolius* the result showed that the adaxial and abaxial leaf epidermal surface showed that *Mallotus oppositifolius* has irregular shaped epidermal cell wall with undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. Also, the result showed that *Mallotus oppositifolius* leaf is hypostomatic which implied that the stomata only occur on the lower surface of the leaf. The result also showed that the stomata of *Mallotus oppositifolius* is anomocytic which implied that Subsidiary cells are absent around the stomata but the guard cells are directly surrounded by epidermal cells. Also, the Transverse section of the leaf of

the three showed upper epidermis, pith, phloem and spongy mesophyll. *Synedrella nodiflora* leaf are irregular in shape with undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. The leaf is hypostomatic (stomata only occur on the lower surface of the leaf. The stomata are anomocytic which implied absence of Subsidiary cells and the guard cells are directly surrounded by epidermal cells. Also, there is presence of unicellular, unglandulartrichome with conical base.

Table 1: Result of leaf powder microscopy of the crude drugs

Parameter	***D. velutinum***	***M. oppositifolius***	***S. nodiflora***
Lignin	Present	Present	Present
Starch	Present	Present	Present
Calcium oxalate	Absent	Present	Absent
Cystoliths	Present	Absent	Present
Tannins	Present	Present	Present
Oil body	Present	Absent	Present

Result of Leaf Microscopy Assays of ***Desmodium velutinum, Mallotus oppositifolius*** **and** ***Synedrella nodiflora***

Leaf Microscopy Assays of ***Desmodium velutinum, Mallotus oppositifolius*** **and** ***Synedrella nodiflora*****.**		

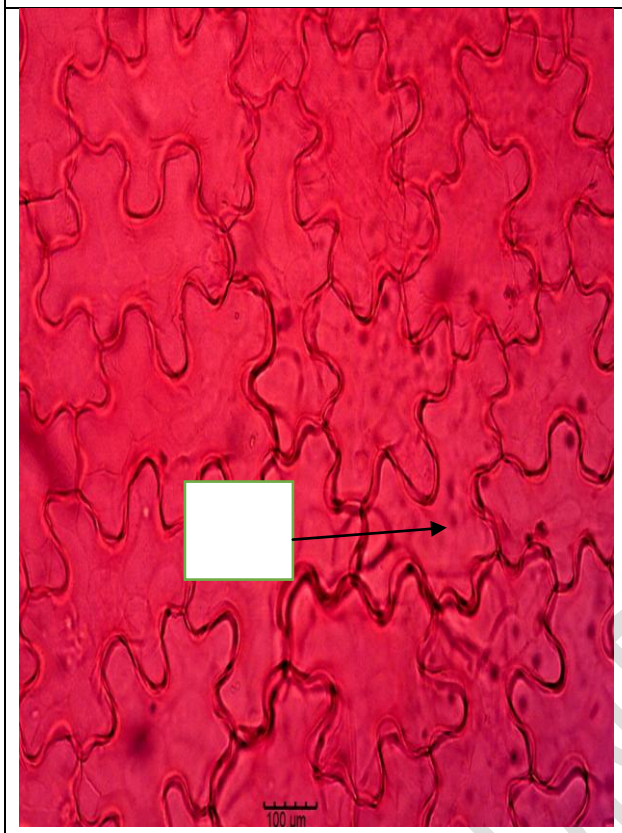
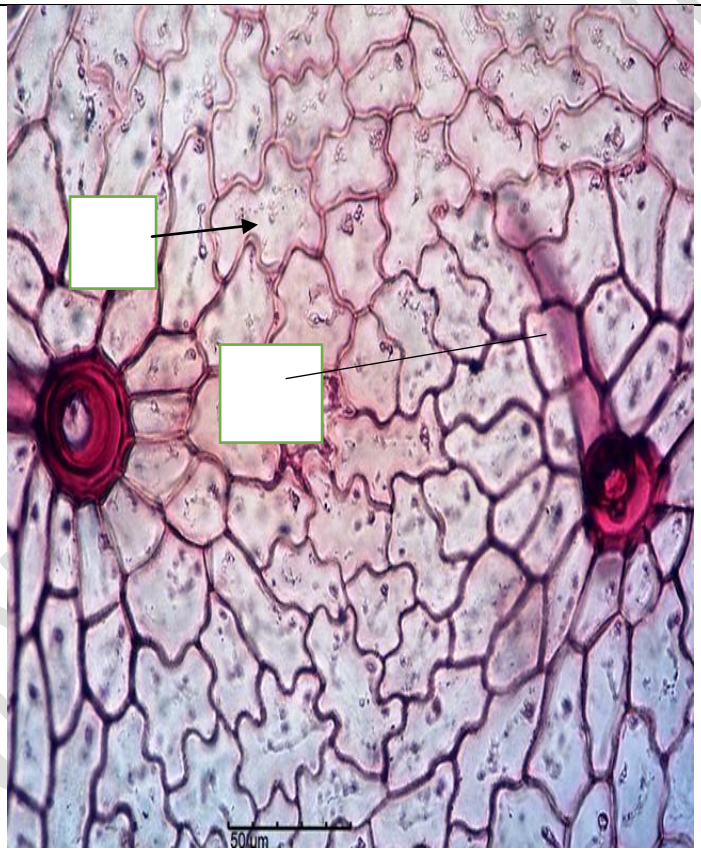
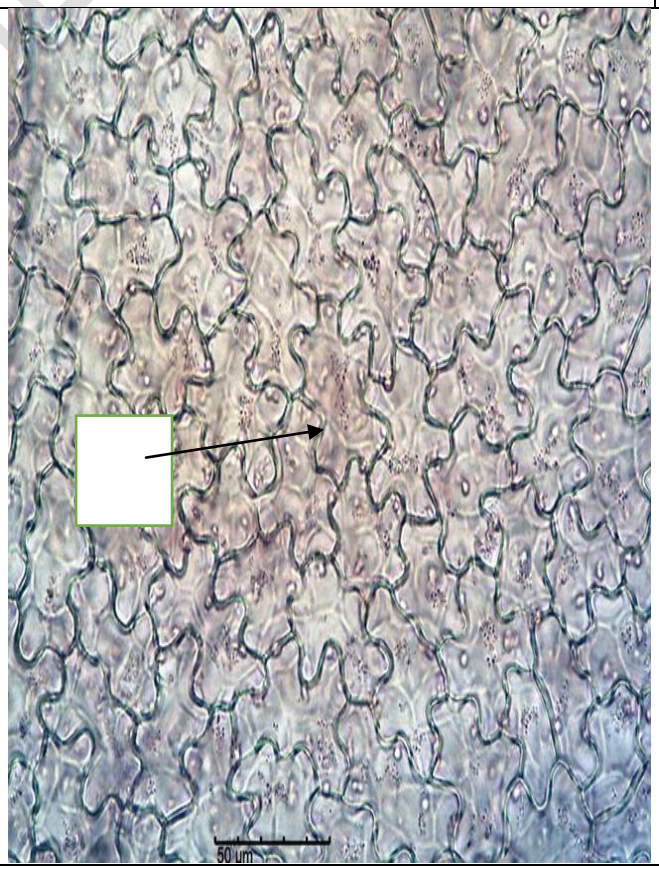

		

Plate 1: Adaxial surface of the leaf of *S.*	Plate 2: Adaxial surface of the leaf of *D. velutinum* showing irregular epidermal cells (IEC) with	Plate 3: Adaxial surface of the leaf of *M. oppositifolius* showing irregular epidermal cells

nodiflora showing irregular epidermal cells (IEC) with undulated/wavy walls. Stomata absent	undulated/wavy walls and presence of unicellular unglandulartrichomes (UUT). Stomata absent	(IEC) with undulated/wavy walls. Stomata absent

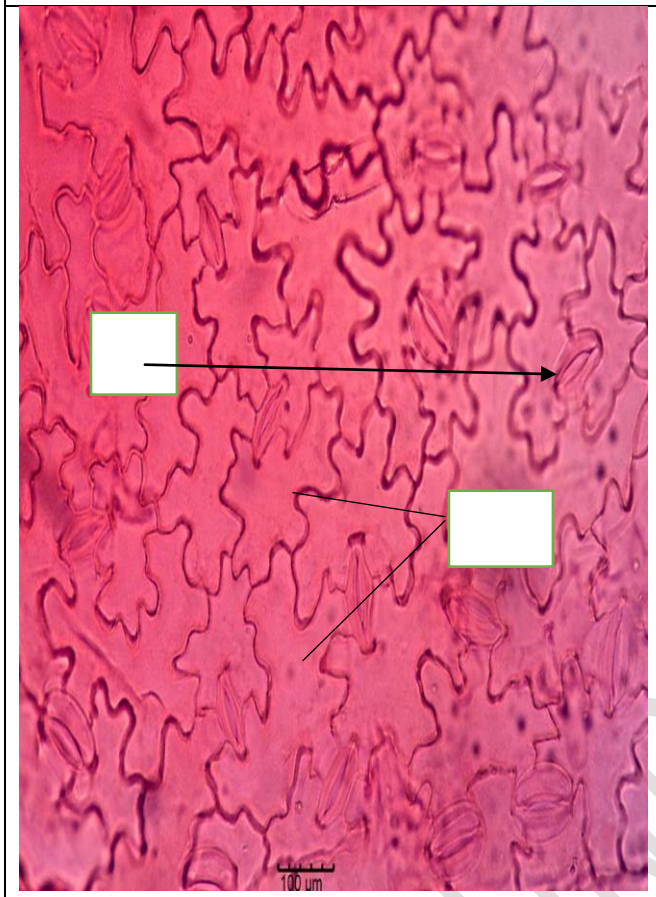
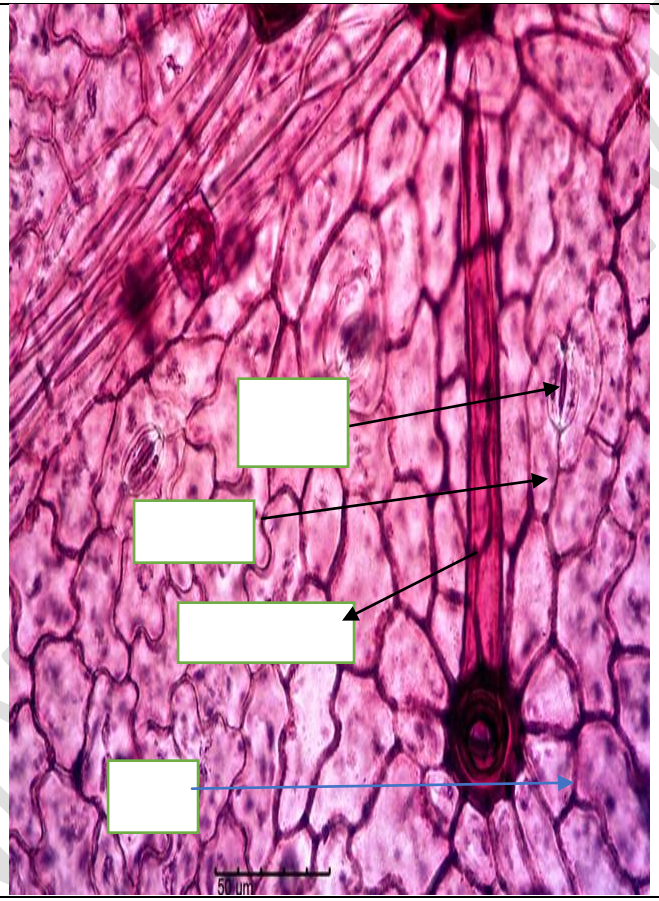
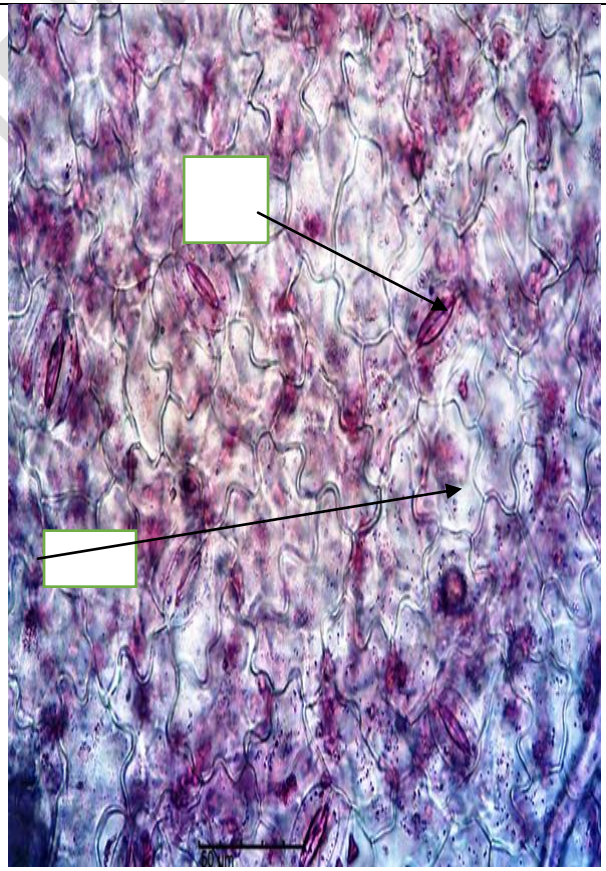

		

Plate 4: Abaxial surface of the leaf of *S. nodiflora* showing anomocytic type of stomata (AS) and irregular epidermal cells (IEC)	Plate 5: Abaxial surface of the leaf of *D. velutinum*showing paracytic type of stomata (PS),	Plate 6: Abaxial surface of the leaf of *M. oppositifolius* showing anomocytic type of stomata

	trichome(T), subsidiary cells (SC) and irregular epidermal cells (IEC)	and irregular epidermal cells

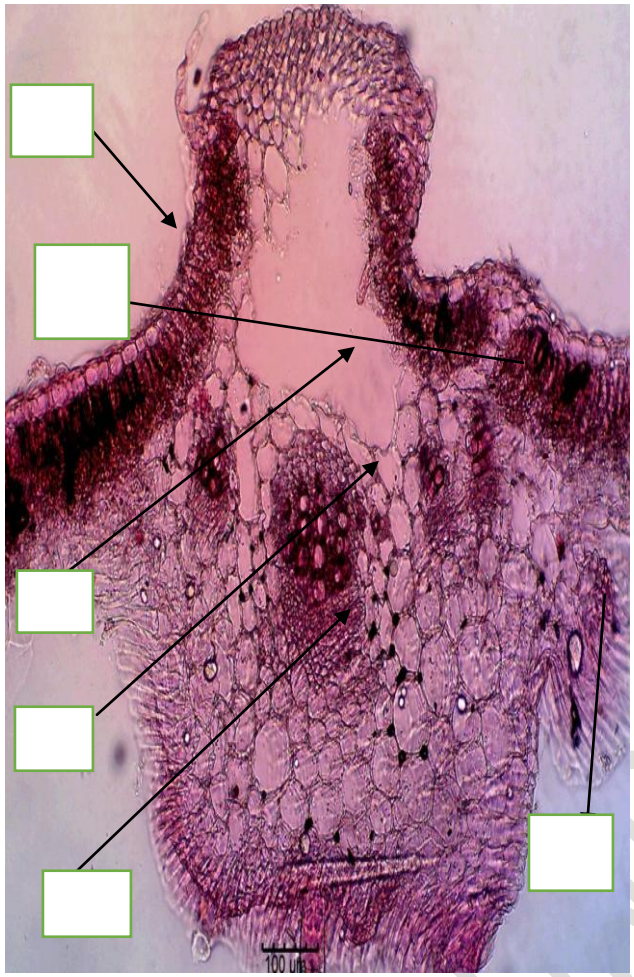
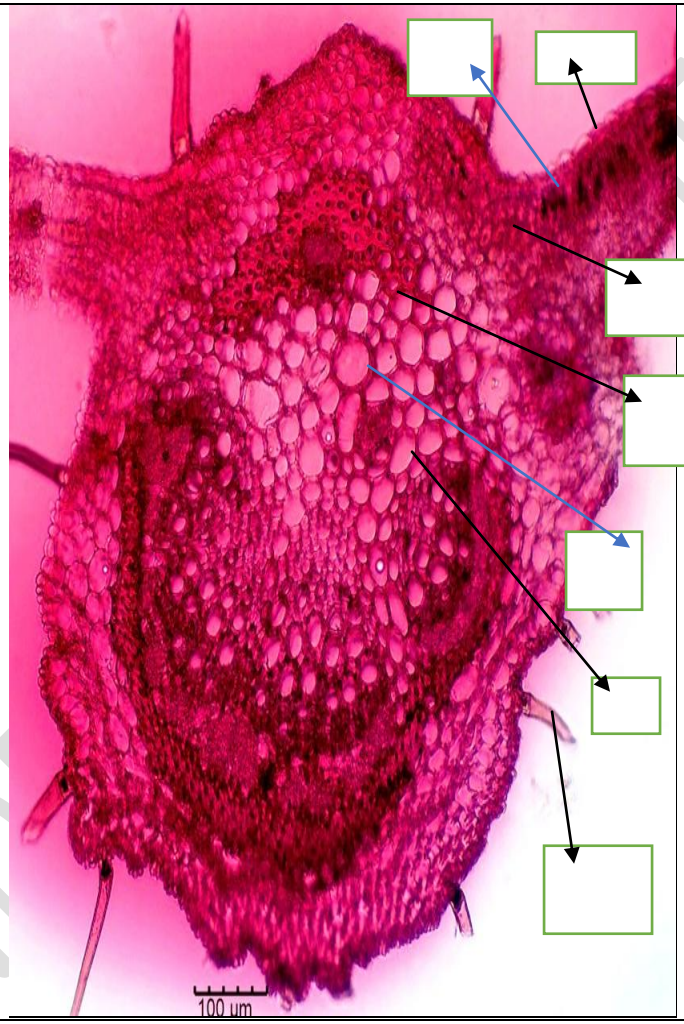
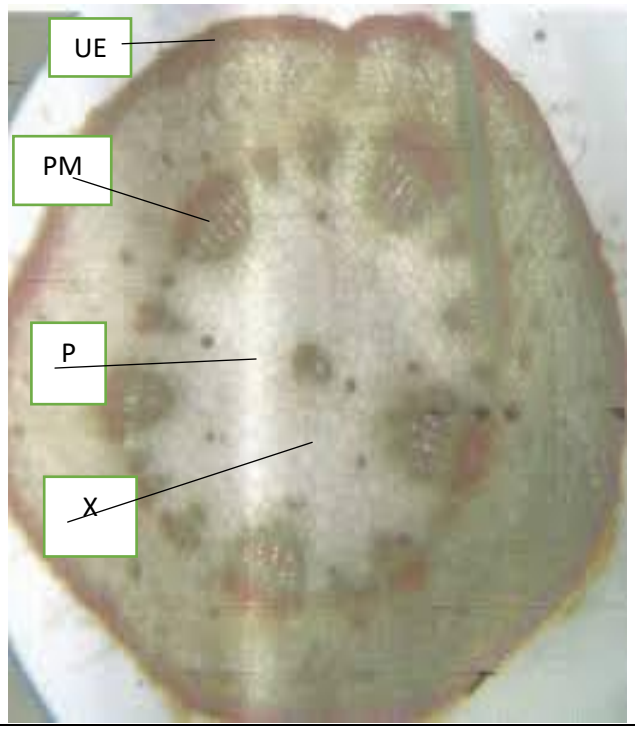

		

Plate 7: Transverse section of the leaf of *S. nodiflora* showing Upper epidermis (UE), spongy mesophyll (SP), Phloem (P), Xylem (X), Pith (Pi) and	Plate 8: Transverse section of *D. velutinum* the leaf of showing Upper epidermis (UE), Palisade Mesophyll (PM) spongy mesophyll (SP), Phloem (P), Xylem (X),	Plate 9: Transverse section of *D. velutinum* the leaf of showing Upper epidermis (UE), Palisade Mesophyll (PM) Phloem (P), Xylem (X) and Pith (Pi)

lower Epidermis (LE).	Trichome and Pith (Pi)	

Chemomicroscopy of D. velutinum, M. oppositifolius and S. nodiflora

The result of the Chemomicroscopy is presented in plate 10 to 13. The result showed that the Chemomicroscopy of S. nodiflora showed the presence of lignin, starch, cystoliths, tannins and oil body while crystal of calcium oxalate was absent in the leaf powder sample (Plate 10). Also, the result showed that the Chemomicroscopy of D. velutinum showed the presence of linin, starch, Cystoliths, Tannins and oil body, crystal of calcium oxalate, trichome and a strand of fibre in the leaf powder sample (Plate 11). Furthermore, the result showed that the chemomicroscopy of M. oppositifolius showed the presence of linin, starch, tannins and crystal of calcium oxalate while oil body and cystoliths were absent in the leaf powder sample (Plate 12).

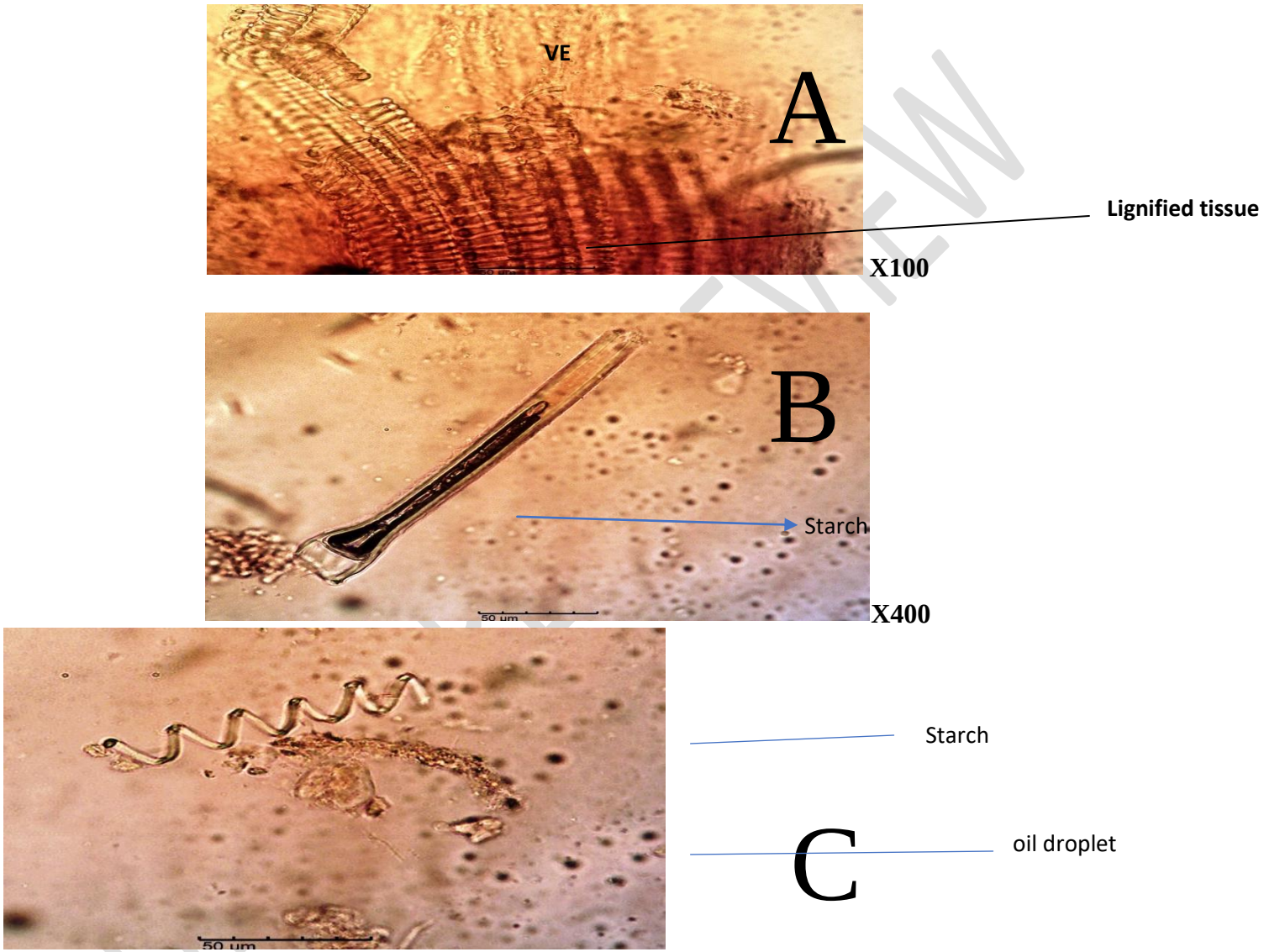


Plate 10: Chemomicrograph of the powdered leaf of *S. nodiflora* (A shoed lignified tissue, B showed starch while C showed oil droplet)

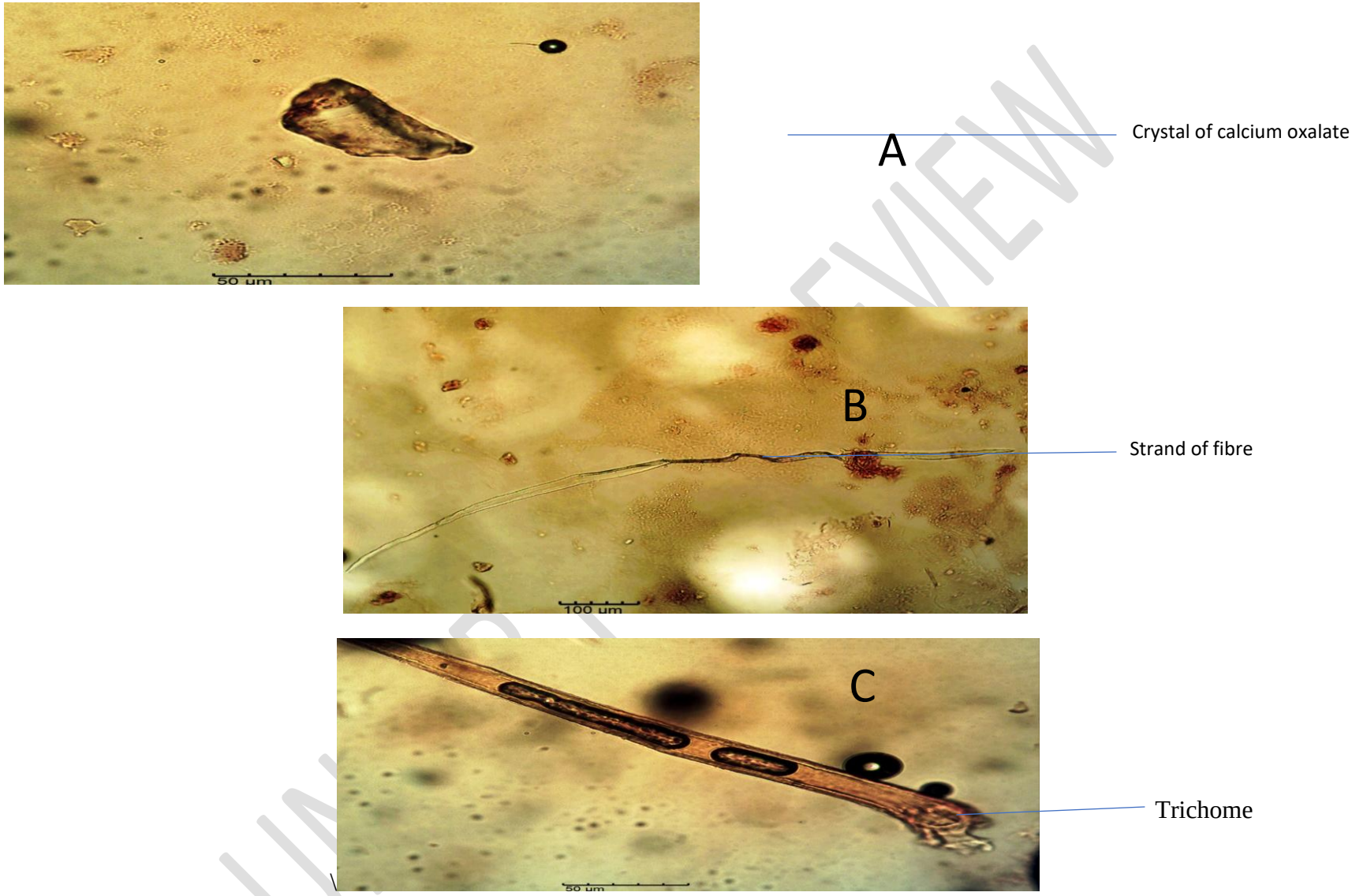


Plate 11: Chemomicrograph of the powdered leaf of *D. velutinum* (A showing crystal of calcium oxalate, B showed strand of fibre while C showed a unicellular covering trichome impregnated with rod-shaped cystoliths.

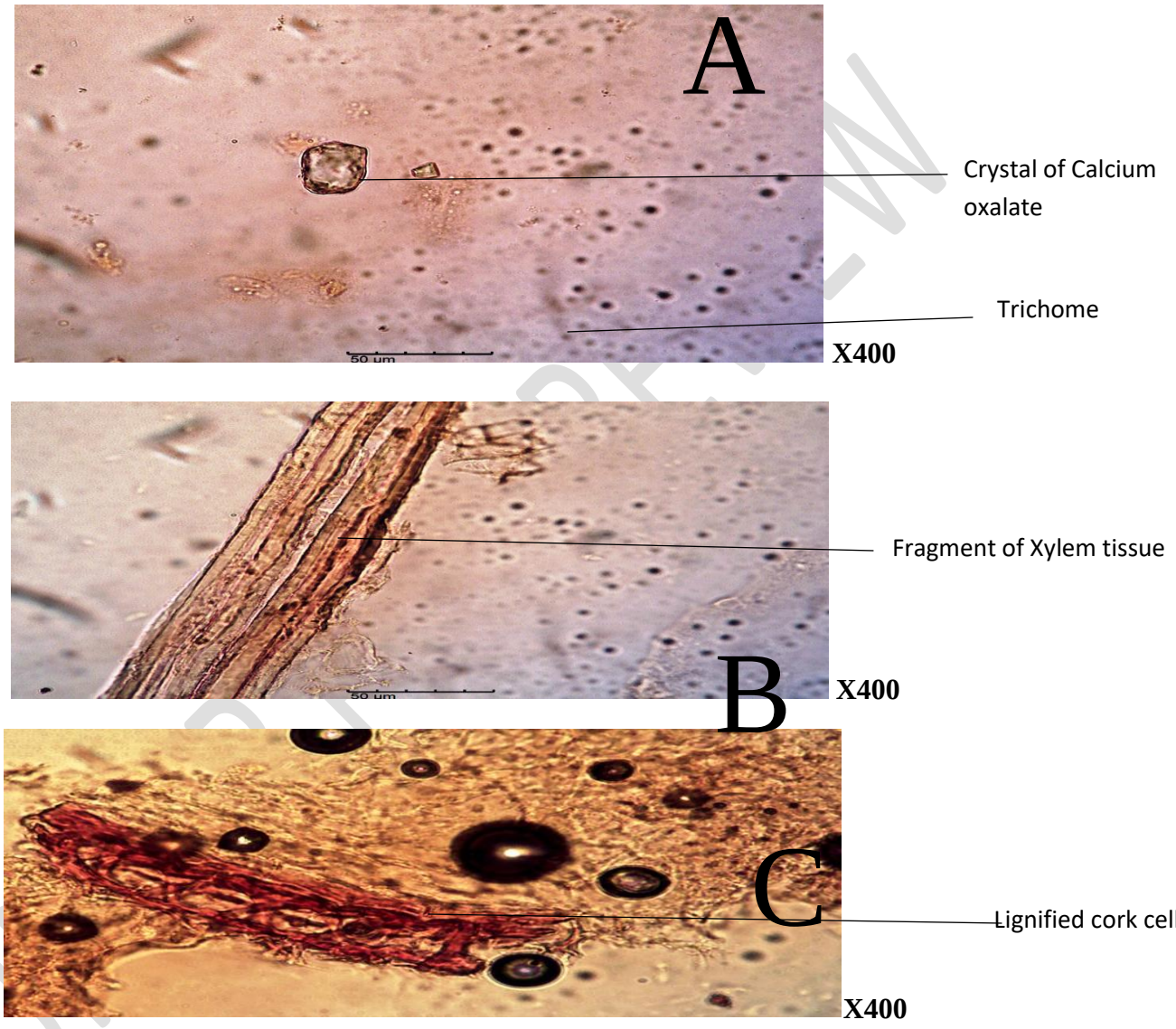


Plate 13: Chemomicrograph of the powdered leaf of M. oppositifolius (A showed trichome and crystal of calcium oxalate, B showed fragment of xylem tissue while C showed lignified tissues)

Quantitative Leaf Characteristics

The result of the quantitative leaf characteristics is presented in Figure 1.0 as follows:

Epidermal cell number

The result of the epidermal cell number is presented in Figure 1.0. The result showed that *D. velutinum* (76) had the highest number of epidermal cells on the surface of the leaf, followed by *M. oppositifolius* (66.25) while *S. nodiflora* (46.75) had the least number of epidermal cells.

Palisade ratio

The result of the palisade ratio is presented in Figure 1.0. The result showed that *S. nodiflora*had significantly high palisade ratio of 11.5 followed by *D. velutinum* (9.25) while *M. oppositifolius* had significant lower palisade value of 8.0.

Stomata Density

The result of the stomata density is presented in figure 1.0. The result showed that *S. nodiflora*had significantly high stomata density of 97 mm^{-2} followed by *M. oppositifolius (*56 mm^{-2}) while *D. velutinum*had significant lower stomata density of 22 mm^{-2}.

Stomata Index

The result of the Stomata Indexis presented in Figure 1.0. The result showed that *S. nodiflora*had significantly high stomata index of 26 % followed by *M. oppositifolius* (13%) while D. *velutinum* had significant lower stomata index of 4.7 %.

Stomata Length

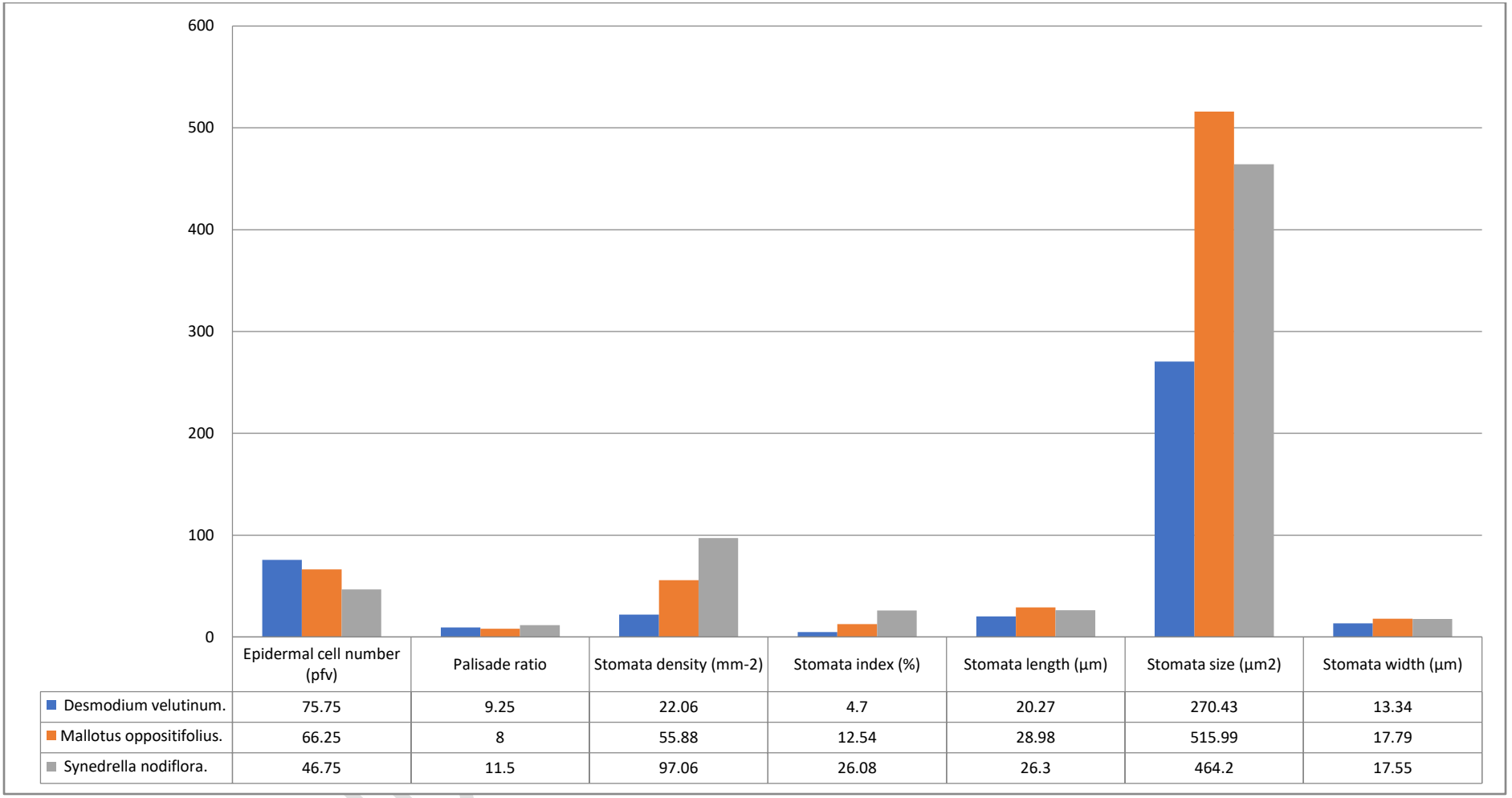
The result of the Stomata Lengthis presented in Figure 1.0. The result showed that *M. oppositifolius* had significantly bigger Stomata lengthof28.98µm, followed by *S. nodiflora*(26.3 µm) while *D. velutinum*had significant smaller stomata length of 20.27 µm.

Stomata Size

The result of the Stomata Sizeis presented in Figure 1.0. The result showed that *M. oppositifolius* had significantly larger Stomata sizeof 516 μm^2, followed by *S. nodiflora*(464 μm^2) while *D. velutinum* had significant smaller stomata size of 270 μm^2.

Stomata width

The result of the Stomata widthis presented in Figure 1.0. The result showed that *M. oppositifolius* had significantly bigger Stomata widthof 17.79µm, followed by *S. nodiflora*(17.55 µm) while *D. velutinum* had significant smaller stomata width of 13.34 µm.



	Epidermal cell number (pfv)	Palisade ratio	Stomata density (mm-2)	Stomata index (%)	Stomata length (µm)	Stomata size (µm2)	Stomata width (µm)
Desmodium velutinum.	75.75	9.25	22.06	4.7	20.27	270.43	13.34
Mallotus oppositifolius.	66.25	8	55.88	12.54	28.98	515.99	17.79
Synedrella nodiflora.	46.75	11.5	97.06	26.08	26.3	464.2	17.55

Figure 1 : Qualitative Characteristics of *D. velutinum*, *M. oppositifolius* and *S. nodiflora* leaves microscopy of the crude drugs.

Physicochemical Assays

The ash value, extractive value, and the RF values of the crude drugs was evaluated for *D. velutinum, M. oppositifolius* and *S. nodiflora* and are presented in Table 2 to Table

Ash Value

The results of the ash value were presented in Table 2. The result showed that total ash was significantly higher in D. *velutinum* (16 %) followed by *M. oppositifolius* (13.6%)but was least in S. *nodiflora* (6.7%). Water soluble ashes were also significantly higher in D. *velutinum* (10 %) followed by *M. oppositifolius* (6.6%)but was least in S. *nodiflora* (3.8%). The result further showed that acid insoluble ash was also significantly higher in D. *velutinum* (7.0%) followed by *M. oppositifolius* (6.4%)but was least in S. *nodiflora* (3.8%). Water loss on drying was however the same (0.9%) across the three crude plants.

Table 2: Ash Value of *M. oppositifolius*, *D velutinum* and *S. nodiflora*

SN	Parameter	Values (Percentage)		
		D. velutinum	***M oppositifolius***	***S nodiflora***
1	Total Ash %	16.010 ± 0.058 [c]	13.633 ± 0.058 [a]	6.713 ± 0.006 [b]
2	Water soluble ash %	10.167 ± 0.058 [c]	6.617 ± 0.058 [b]	3.833 ± 0.058 [a]
3	Acid Insoluble ash	7.033 ± 0.058 [c]	6.467 ± 0.058 [b]	3.833 ± 0.058 [a]
4	Water loss on drying	0.990 ± 0.010 [a]	0.940 ± 0.000 [a]	0.940 ± 0.000 [a]

Note: Mean with the same alphabet are not statistically different while mean with different alphabets are statistically different.

Extractive Value

The result of the extractive value of *D. velutinum*, *M. oppositifolius* and *S. nodiflora* using water, ethanol, methanol and hexane was presented in Table 3. The result showed that greater yield of extract for *D. velutinum* was obtained with water ((10.8%)followed by methanol (9.6%) and ethanol (7.2%) but was least in hexane (2.2%).

The highest extract yield for *M. oppositifolius* was obtained with using methanol (10.6%) followed by aqueous extract (9.8%) and ethanol (5.9%) but was least with hexane. Also, the result further reveled that methanol (15.3%) gave greater yield of crude extract for *S. nodiflora* followed by aqueous (13.6%) and ethanol (2.9%) while hexane (2.60) had the lowest yield.

Table: 3 : Extractive Values of *M. oppositifolius, D velutinum and S nodiflora*

SN	Solvent	Values (Percentage)		
		D velutinum	***M oppositifolius***	***S nodiflora***
1	Water %	10.833 ± 0.058^{d}	9.7667 ± 0.058^{c}	13.633 ± 0.058^{c}
2	Methanol %	9.633 ± 0.058^{c}	10.567 ± 0.058^{d}	15.267 ± 0.058^{d}
3	Ethanol %	7.233 ± 0.058^{b}	5.900 ± 0.100^{b}	2.900 ± 0.100^{ab}
4	Hexane %	2.233 ± 0.058^{a}	3.403 ± 0.058^{a}	2.600 ± 0.100^{a}

Thin Layer Chromatography of *M. oppositifolius*, *D. velutinum* and *S. nodiflora*

The result of the tin layer chromatography using methanol and ethyl acetate solvent in the ratio of 6:4 for *M. oppositifolius, D velutinum and S nodiflora*is presented in Table 4 as follows: *M. oppositifolius* had 3 visible spot with retention factor (RF) of 0.363, 0.425 and 0.5 in that order respectively, *D. velutinum* had 4 visible spot with

retention factor (RF) of 0.425, 0.5, 0.550 and 0.66 in that order respectivelywhile *S. nodiflora* had 6 visible spot with retention factor (RF) of 0.255, 0.363, 0.438, 0.538, 0.600 and 0.688 in that order respectively.

Table 4: RF Values of *M. oppositifolius, D. velutinum* and *S. nodiflora*

SN	**RF Values**		
	D. velutinum	***M. oppositifolius***	***S. nodiflora***
No of visible Spot	4 spots	3 spots	6 spots
1	0.425	0.363	0.255
2	0.500	0.425	0.363
3	0.550	0.500	0.438
4	0.663	-	0.538
5	-	-	0.600
6	-	-	0.6875
(Solvent = Methanol +ethyl acetate) @ ratio of 6: 4			

Phytoconstituents

Phytochemical constituents of *D. velutinum, M. oppositifolius,* and *S. nodiflora* studied were investigated for the following metabolites: alkaloid, flavonoids, anthraquinones, steroids, terpenoids, proteins, carbohydrates, anthocyanins, Saponin, tannin and reducing sugar. The results of photochemical analysis were presented in both Tables 5 and 6.

The qualitative analysis was presented in Table 5 while the quantitative analyses were presented in Table 6.

Qualitative Phyto-chemical Analysis

The result of the qualitative phytochemical is presented in Table 5.0. All the phytochemicals tested were present in honey however, the result further showed presence of Alkaloids, Glycosides, Flavonoids, Tannins, Phlobatanins, Saponins, Proteins, Carbohydrates and Anthocyanins across the three plants *D. velutinum, M. oppositifolius, S. nodiflora*. Anthraquinones and steroids were present in *M. oppostifollous* but were absent in *D. velutinum* and *S nodiflora* while terpenoids was present in *D. velutinum* but absent in both *M. oppositifolius* and *S. nodiflora*. The result further showed the presence of reducing sugar in *S. nodiflora* but was absent in *D. velutinum* and *M. oppositifolius*

Table: 5 The Qualitative Phytochemical constituents of *M. oppositifolius,* *D velutinum and S nodiflora*

SN	Bioactive compound	*D. velutinum*	*M. oppositifolius*	*S. nodiflora*
1	Alkaloids	Present $^{++}$	Present $^{+}$	Present $^{+}$
2	Glycosides	Present $^{+}$	Present $^{+}$	Present $^{+}$
3	Flavonoids	Present $^{++}$	Present $^{++}$	Present^{++}
4	Tannins	Present $^{+++}$	Present $^{+}$	Present $^{+++}$
5	Phlobatanins	Present $^{++}$	Present $^{++}$	Present $^{++}$
6	Saponin	Present $^{++}$	Present $^{+++}$	Present $^{++}$
7	Steroid	**Absent** $^{(-)}$	Present $^{++}$	**Absent** $^{(-)}$

8	Proteins	Present $^{++}$	Present^{++}	Present $^{++}$
9	Terpenoids	Present $^{+}$	**Absent** $^{(-)}$	**Absent** $^{(-)}$
10	Carbohydrates	Present $^{++}$	Present $^{+}$	Present $^{++}$
11	Anthraquinones	**Absent** $^{(-)}$	Present $^{+}$	**Absent** $^{(-)}$
12	Anthocyanins	Present $^{++}$	Present $^{+}$	Present $^{++}$
13	Reducing Sugar	**Absent** $^{(-)}$	**Absent** $^{(-)}$	Present^{++}

Qualitative Phyto-chemical Analysis

The result of the pharmacologically important quantitative phytochemical assay is presented in Table 6 and it showed that *D. velutinum*, *M. oppositifolius*, *S. nodiflora* contained varying amount of these therapeutically important phytochemicals.

The result showed that *D. velutinum* has the highest content of Alkaloid (13.7), and Tannins (17.85) followed by *M. oppositifolius* (7.97 & 17.72) and S. nodiflora (5.46 & 11.573) respectively in that order while honey has the least content of Alkaloids (0.224) and Tannins (0.67) respectively.

Flavonoid content was highest in *D. velutinum* (60.29), followed by *M. oppositifolius* (28.21)) while *S. nodiflora* was the least (2.75). Saponin was highest in *M. oppositifolius* (0.24)followed by *D. velutinum* (0.23) respectively while S. nodiflora (0.14) had the least. Steroids content were higher in *M. oppositifolius* (0.077) followed by *D. velutinum* (0.049) but was absent in *S. nodiflora*. Terpenoids were absent in *M. oppositifolius* and *S. nodiflora* but was present in *D. velutinum* (0.48). Carbohydrates were highest in *D. velutinum* (36.49) followed by *M. oppositifolius* (33.79) while in *S. nodiflora* (21.11) it was the least. Reducing sugar was highest in D. *velutinum* (6.84) and *S. nodiflora* (2.01) respectively but was least in *S. nodiflora* (1.65) respectively.

Table: 6 The Quantitative Phytochemical constituents of *M. oppositifolius*, *D velutinum*and*S nodiflora*

SN	Bioactive compound	*D velutinum*	*M oppositifolius*	*S nodiflora*
1	Alkaloids	13.740 ± 0.052 [d]	7.797 ± 0.023 [c]	5.457 ± 0.006 [b]
2	Flavonoids	60.290 ± 0.017 [d]	28.213 ± 0.029 [c]	2.747 ± 0.006 [a]
3	Tannins	17.850 ± 0.017 [d]	17.723 ± 0.029 [c]	11.573 ± 0.029 [b]
4	Saponins	0.228 ± 0.002 [b]	0.239 ± 0.003 [c]	0.143 ± 0.004 [a]
5	Steroid	0.049 ±0.002 [a]	0.077 ±0.000 [b]	-
6	Terpenoids	0.483 ±0.001 [a]	-	-
7	Carbohydrates	36.486 ± 0.029 [c]	33.793 ± 0.046 [b]	21.113 ± 0.029 [a]
8	Reducing sugar	6.837 ± 0.029 [c]	2.013 ± 0.029 [b]	1.65 ± 0.017 [a]

Note: Mean with the same alphabet are not statistically different while mean with different alphabets are statistically different.

Acute toxicity studies

The result of the acute toxicity studies showed that LD_{50} of *M. oppositifolius, D velutinum and S nodiflora* were above 5000mg/kg as presented in Table 7. These implied that the three plants are relatively safe.

Table 7. Acute toxicity test of *M. oppositifolius, D velutinum and S nodiflora*

Phase	Dose (Mg/kg)	No of Animals used	Death Ratio		
			D velutinum	***M oppositifolius***	***S nodiflora***
Phase 1	10	3	0/3	0/3	0/3
	100	3	0/3	0/3	0/3
	1000	3	0/3	0/3	0/3
Phase 2	1200	1	0/1	0/1	0/1

	1600	1	0/1	0/1	0/1
	2900	1	0/1	0/1	0/1
	5000	1	0/1	0/1	0/1

Note: 0/3 and 0/1 means that no animal died when 3 and 1 animals were used respectively:

The Result implied that the LD_{50} of *M. oppositifolius, D velutinum and S nodiflora is above 5000mg/ kg body weight of mice*

Discussion.

Pharmacognostic parameters using stomata stomatal parameters revealed a number of important exhibited interesting variations among the three families studied and this implied that this could be a strong diagnostic tool for classification and identification of crude drug. This is a vital tool in classification and identification of crude drugs. There was a wide variation in the number and distribution of stomata found in all the three-family studied. Whereas, Desmodium velutinum (Fabaceae) had the least stomata size (length and width), stomata density and stomata index, it has the highest number of cells and this could be an adaptation to conserve water in the species of the family. M. oppositifolius (Euphorbiaceae) on the other hand had the highest size (length and width), it has the least palisade ratio while S. nodiflora (Asteraceae) had the highest density and stomata index and this could be a key in their adaptation to different environmental stress.

The differences reported in the quantitative parameters of the leaf (stomatal size, cell wall thickness and stomata index) could be attributed to their physiological responses to a number of environmental factors and these differences could be usefully adopted as diagnostic characters in their delimitation at the family genus and specie

level [28-29]. hence very useful in identification of the plants. The present study provided the pharmacognistic, physicochemical, phytochemical and toxicological details of the *D. velutinum, M. oppositifolius, S. nodiflora* leaf which are useful in laying down standardization and pharmacopoeia parameters.

References

1. Kunle, O.F., Egharevba, H. O. and Ahmadu, P.O. (2012). Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation Vol. 4(3), pp. 101-112. DOI: 10.5897/IJBC11.163
2. WHO (2005). WHO Global Atlas of Traditional, Complementary and Alternative Medicine. World Health Organization, Geneva. 1and 2.
3. WHO (2002). WHO Monographs on Selected Medicinal Plants, World Health Organization, Geneva. 2.
4. WHO (1999a). Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva.
5. WHO (1999b). WHO Monographs on Selected Medicinal Plants, World Health Organization, Geneva. 1.
6. WHO (1990). The Use of Essential Drugs. Eighth report of the WHO Expert committee. World Health Organization, Geneva.
7. Blumenthal M (2000) Ephedra update: industry coalition asks FDA to adopt national labeling guidelines on ephedra; offers co-operative research with NIH. Herbal Gram., 50: 64-65.
8. Blumenthal M, Brusse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW, Rister RS (1998). The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines. The American Botanical Council, Austin, TX.
9. Bisset NG (1994). Herbal Drugs and Phytopharmaceuticals. Medpharm GmbH Scientific Publishers, Stuttgart, CRC Press, Boca Raton, 91-95.
10. Mashile, S.P. and M.P. Tshisikhawe. 2017. Epidermal structure of stomata and trichomes of Vachelliatortilis (Forssk.) Galasso and Banfi. Pak. J. Bot., 49(6): 2353-2355.
11. Sonibare, M.A. and A.A. Adeniran. 2014. Comparative micromorphological study of wild and micropropagatedDioscoreabulbiferaLinn. Asian Pacific J. Trop. Biomed., 4(3): 176-183.

12. Anowi C.F.,Onyegbule A.F., Onyekaba E., Azode C. Antipyretic and phytochemical evaluation of the ethanol extract of the leaf of *Desmodium velutinum*. Asian J. Pharm. Life Sci., (2012) 2: 135-143.
13. **Rathi MJ and Gopalkrishnan S. 2005.** Insecticidal activity of aerial parts of *Synedrella nodiflora* (L.) Gartn (Compositae) on *Sapodepteralatura*(FAB). *J.cent.Eur.Agric.* **6**: 323-328.
14. Nwaehujor C.O., Ezeigbo I.I. and Nwinyi F. C (2013). Evaluation of Mallotus oppositifolius Methanol Leaf Extract on the Glycaemia and Lipid Peroxidation in Alloxan-Induced Diabetic Rats: A Preliminary Study. Biochem Res Int'l. 2013; doi.org/10.1155/2013/527205.
15. Adekunle A.A and Ikumapayi A.M. (2006). Antifungal Property and Phytochemical Screening of the Crude Extracts of FuntumiaElastica and Mallotus Oppositifolius. West Indian Med J. 2006;55(4):219-23.
16. Fabricant D.S, and Farnasworth N. R. (2001). The value of plants used in traditional medicine for drug discovery. Environ. Health Prospect Suppl. 2001; 109 (1): 69-76.
17. Nwafor F.I., NwosuM.O ., Nwafor A.Z. (2019). Taxonomic and Ecological Significance of Foliar Epidermal Characters in Four Taxa of *Mussaenda L*. (Rubiaceae) in Nigeria. *Annual Research & Review in Biology*, 32(5): 1-12.
18. Johansen D. A. (1950). *Plant Microtechnique*. McGraw-Hill Book Company, New York, pp 230.
19. Nwosu M. O. (2006). Preparation of Botanical Slides. In: Inyang, N. M., Nwosu, M. O. and Ivoke, N. (eds). *Manual of Laboratory Techniques in Biology*. University of Nigeria Press Ltd Nsukka, Nigeria, p. 131 – 163.
20. Sofowora A. (1993). Medicinal plants and traditional medicines in Africa. Nigeria, Lagos: Spectrum books limited pp. 55-61.
21. Harbone J.B., Mabry T.J. and Mabry H (1975). The Flavonoids, 1st ed.; Springer SV Media: Austin, TX, USA.
22. Koparde A.A, Doijad C.R. and Magdum C.S. (2019). Natural Products in Drug Discovery, Pharmacognosy - Medicinal Plants, ShaguftaPerveen and Areej Al-Taweel, IntechOpen, DOI: 10.5772/intechopen.82860. Available from: https://www.intechopen.com/chapters/65128
23. SingletonV. L., and Rossi, J. A. **(1965).** Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American journal of Enology and Viticulture vol16 (1965): 144-158.
24. Marinova D., Ribarova F. and Atanassova, M. (2005). Total Phenolics And Total Flavonoids in Bulgarian Fruits and Vegetables. Journal of the University of Chemical Technology and Metallurgy volume 40 (3) (2005), 255-260.
25. Ghorai N., Chakraborty, S., Gucchait S., Saha K S. and Biswas B., Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. Nature Protocol Exchange (2012). https://doi.org/10.1038/protex.2012.055

26. Lorke D. (1983) A new approach to practical acute toxicity testing. *Archives Toxicology* 54: 279-287.https://doi.org/10.1007/bf01234480.
27. Onyeka I.P, Suleiman M.M., Bako S.P. (2018). Toxicity Effects of Methanolic Extract of *Euphorbia hirta*-Honey Mixture in Albino Rats. J Pharmacogn Nat Prod 4: 147. Doi: 10.4172/2472-0992.1000147.
28. Adegbite, A.E. 2008. Leaf anatomical studies in some species of the tribe Cichoricae (Asteraceae) in Nigeria. Comp. Newsl., 46: 49-58.
29. Aworinde, D.O., B.O. Ogundairo, K.F. Osuntoyinbo and O.A. Olanloye. 2012. Foliar epidermal characters of some Sterculiaceae species in Nigeria. Bayero J. Pure Appl. Sci., 5(1): 48-56.